PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Thomas D. Madden et al.
Application No.	:	09/896,812
Filed	:	June 29, 2001
For	:	LIPOSOMAL ANTINEOPLASTIC DRUGS AND USES THEREOF

Examiner	:	Gollamudi S. Kishore
Art Unit	:	1615
Docket No.	:	480208.408

DECLARATION OF THOMAS D. MADDEN, Ph.D.
PURSUANT TO 37 C.F.R. §1.132

I, Thomas D. Madden, Ph.D. declare as follows:

1. I currently hold the position of Senior Director, Technology Development and Licensing, at Inex Pharmaceuticals, located in Burnaby, British Columbia, Canada, the assignee of the above-identified application (the "application"), and I am a co-inventor of the subject matter disclosed therein.

2. I am familiar with the content of the application, and I have reviewed the Office Action mailed October 3, 2005 and the prior art references cited therein, namely U.S. Patent No. 6,110,491 ("Kirpotin") and U.S. Patent No. 5,543,152 ("Webb"). In this Office Action, the Examiner alleges that the claimed liposomal vinorelbine formulations are obvious over these references. I submit this Declaration further to a personal interview conducted with the Examiner on December 7, 2006, in order to provide additional evidence that the liposomal vinorelbine formulations claimed in the instant application are not obvious in light of these references. In addition, I provide materials discussed during the personal interview, which demonstrates that the

claimed high drug:lipid liposomal vinorelbine formulations surprisingly have enhanced drug retention properties.

3. I submit that the presently claimed liposomal vinorelbine formulations, which have a drug:lipid ratio in the range of 0.2-0.3:1 (w/w) were surprisingly found by Applicants to have enhanced drug retention *in vivo* as compared to liposomal vinorelbine formulations at lower drug:lipid ratios. This contravenes the conventional wisdom in the art, which was that higher drug:lipid ratios lead to increased drug leakage from liposomes. As discussed during the interview, it is understood in the art that drug uptake that occurs during pH gradient loading of liposomes depletes the ion gradient. Therefore, lower drug:lipid ratios result in a higher residual gradient, while higher drug:lipid ratios result in a lower residual gradient. Since this residual gradient maintains vinorelbine in its protonated form, thereby preventing its leakage from the liposome interior, it follows that lower drug:lipid ratios would be expected to be more effective in retaining vinorelbine within the liposome. Higher drug:lipid ratios, with a corresponding lower residual ion gradient, would be expected to allow an increased amount of vinorelbine to leak from the liposomes. This phenomenon is depicted in Attachment A. Consistent with these theoretical considerations, pharmacokinetic studies conducted by INEX on liposomal formulations of vincristine loaded at differing drug:lipid ratios using a citrate gradient show faster drug release from formulations prepared at higher drug:lipid ratios (0.24:1 and 0.14:1) compared to a formulation at low drug:lipid ratio (0.08:1). In light of this understanding in the art and the pharmacokinetic data for liposomal vincristine, it was surprisingly discovered by the inventors of the application that higher vinorelbine:lipid ratios resulted in increased drug retention *in vivo*, as shown in Figure 1 of the application.

4. Upon further investigation at INEX it was discovered that at high drug:lipid ratios vinorelbine precipitates within the liposome interior. As shown in Attachment B, cryogenic-transmission electron microscopy performed on empty sphingomyelin:cholesterol liposomses (A) and vinorelbine-loaded

sphingomyelin:cholesterol liposomes (B) revealed the presence of electron dense amorphous precipitates within the vinorelbine-loaded liposomes. The precipitated vinorelbine must dissolve in the buffer within the liposome interior before it can leak out of the liposomes. Therefore, there is less drug leakage in high drug:lipid ratio liposomal vinorelbine formulations having a significant amount of precipitated vinorelbine.

5. The claimed liposomal vinorelbine formulations also have superior drug delivery and therapeutic properties. Experiments performed in animal models of human breast and colon tumors demonstrated that liposomal vinorelbine formulations having a 0.3:1 (w/w) drug:lipid ratio and sphingomyelin:cholesterol molar ratio of 55:45 provide as much as ten-fold higher levels of vinorelbine to tumor sites as compared to free vinorelbine. The results of these experiments are shown in Attachment C. In addition, the same liposomal vinorelbine formulations exhibited substantial antitumor activity. Experiments performed using HT-29 colon xenografts demonstrated that this liposomal vinorelbine formulation was approximately four times as active as free vinorelbine in reducing and preventing tumor growth. The results of these experiments are shown in Attachment D.

6. In conclusion, I submit that the claimed liposomal vinorelbine formulations provide surprising advantages over the prior art, including enhanced drug retention. These advantages correlate to superior pharmacokinetic and antitumor properties, and would not have been expected in view of the prior art understanding, which was that high drug:lipid ratios result in increased drug leakage.

I hereby declare that all statements made herein are, to my own knowledge, true and that all statements made on information or belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the captioned patent application or any patent issued therefrom.

Date December 19th 2006

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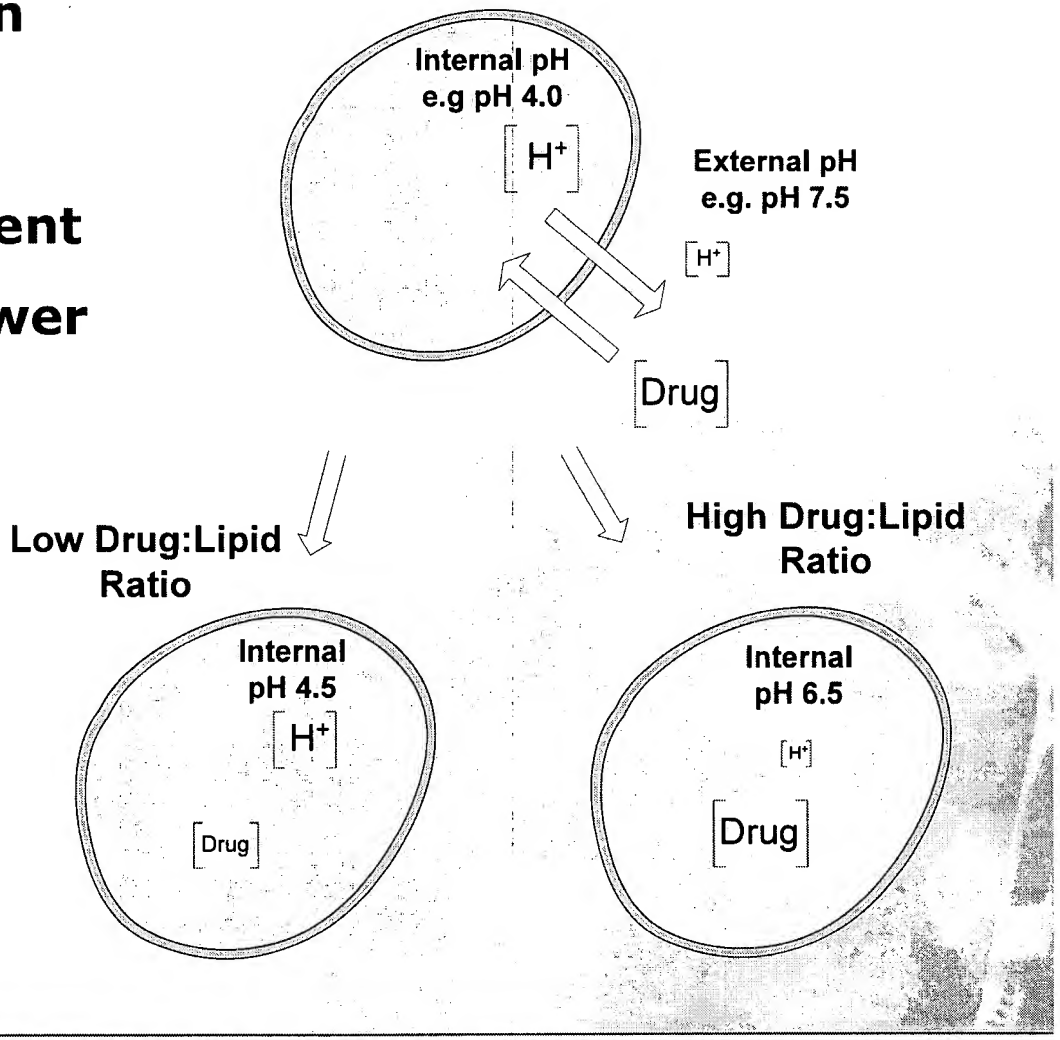
Liposomal Vinorelbine: Influence of drug loading on residual ion gradient

Drug Uptake depletes ion gradient.

- **Lower D:L ratio = higher residual gradient**
- **Higher D:L ratio = lower residual gradient**



Liposomal Vinorelbine: Drug precipitation at high drug:lipid ratios

Slower vinorelbine release rates at high drug:lipid ratios results from drug precipitation within the liposomes

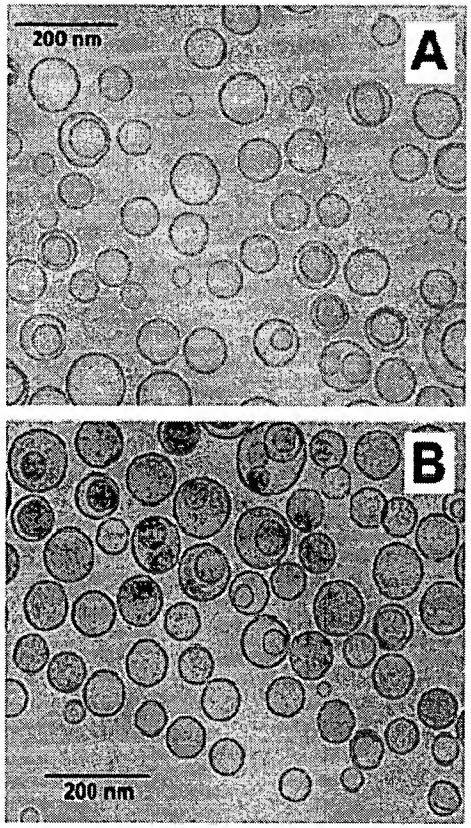
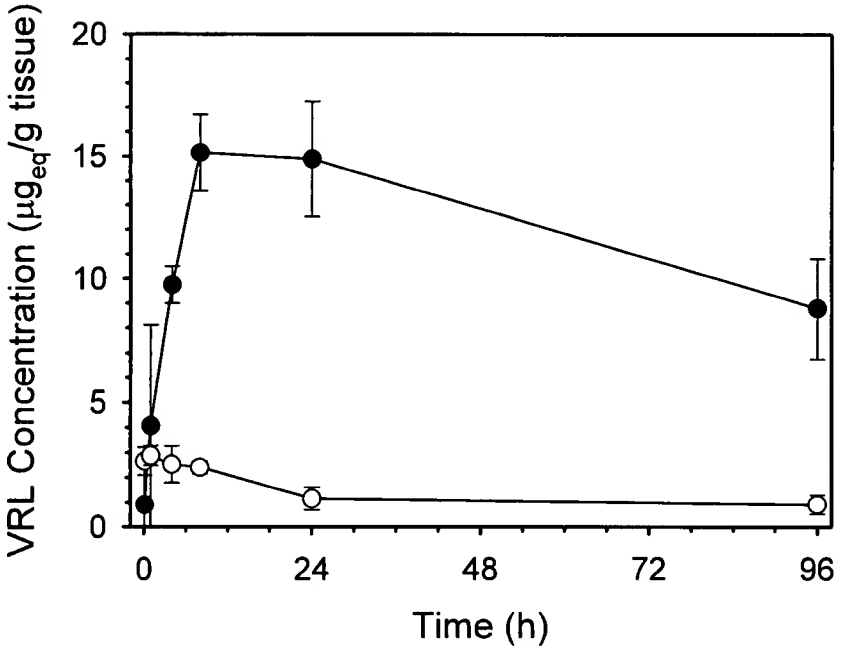


Figure 5. Cryo-electron microscopy of liposomal VRL. Cryogenic transmission electron microscopy (cryo-TEM) was performed on empty SM/Chol liposomes (panel A; 450 mM internal magnesium sulfate) and a drug-loaded SM:Chol formulation containing VRL (panel B; final D/L ratio, 0.25, wt/wt). The micrographs reveal the presence of electron dense amorphous precipitates (darker intra-vesicular regions) in panel B. Magnification is 100000×.

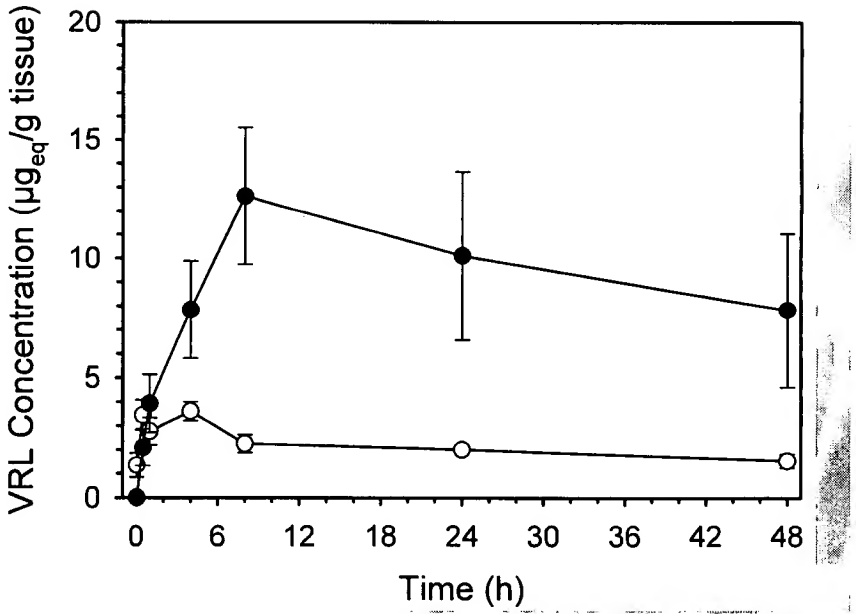
Liposomal Vinorelbine: Increased drug delivery to tumors

Up to 10-fold increase in vinorelbine delivery to tumors with INX-0125 compared to free drug

MX-1 Breast Tumor



HT-29 Colon Tumor



Liposomal Vinorelbine: Antitumor Activity in HT-29 Colon Xenograft

INX-0125 approximately 4-times as active as Vinorelbine